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Macroscopic evaluation of wound healing activity of the Persian shallot, *Allium hirtifolium* in rat

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Abstract

Wound is a break in the outer layer of skin. There are several different methods to provoke wound healing process, consisting topically or orally administration of medicinal drugs or herbal remedies. Persian shallot, *Allium hirtifolium* which belongs to the same genus of garlic, naturally grows in different parts of Iran. In this study, we have avaluated the wound healing activity of hydro-alcoholic extract of *A .hirtifolium* Boiss. Four bilateral full-thickness wounds (2 on each side) were made on the dorsal area of four adult albino rats weighing 165±35 gr under general anesthesia. Right side wounds were treated in experimental groups, while left side wounds were considered as control littermates. One day after surgery, a gel-like 1:1 mixture of *A. hirtifolium* extract and methylcellulose were topically applied (100 mg/kg/day) to the experimental wounds while the wounds in control groups were treated with the extract-free gel for 12 days. At days 1, 3, 6, 9 and 12, digital photos of wounds were taken using of Scion image software, for analyzing the percentage of wound contraction, epithelialisation and speed of healing. The results revealed that *A. hirtifolium* can accelerate wound healing by increasing the rate of epithelialisation. We concluded that *A. hirtifolium* extract may be clinically useful in management of open wounds treatment procedures.

Keywords: *Allium hirtifolium*, epithelialisation, extract, wound healing

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Introduction

The repair or recovery process of wound, after a trauma or a surgical incision is called wound healing. The wound healing process has multiple complicated stages of cellular and biochemical events in response to any disruption of normal anatomy, leading to structural and functional restoration of damaged tissue (Martin, 1996; Tanya and Martin, 2009). There are several reports indicating that herbal medicines possess pro-healing effects. They may accelerate or promote wound healing by their different properties such as encouraging blood clotting, promoting the expression and release of growth factors and cytokines, or even via antibacterial effects.

Pharmacological effects of many herbal remedies had been observed on humans long before their mechanism of action being discovered. Based upon thousands of years of experience, herbal drugs and their derivatives are the main sources of many conventional drugs and provide potential alternative to modern medicine. Therefore their effectiveness, structure, properties and their proper doses should be identified and formulated for different purposes such as management or treatment of wounds (Salunkhe and Kadam, 1998; Phillipson, 2001). In each culture, natural plants with pharmacological effect outlines the traditional herbal medicine. These collections has to scientifically get investigated for their therapeutic potential and possible discovery of new drugs (Hostettmann et al., 1998). Due to the wide range of climatic conditions, Iran having more than 2,000 plant species with great theraputic potential (Rechinger, 1984; Ashrafi et al., 2004), holds a natural rich diversity of herbal flora. Although several reports indicated the antibacterial, anti-inflammatory and wound healing activity of iranian herbs, but the vast majority has not been investigated yet (Ghahreman, 1984; Gupta and Rustgi, 2004).

A. hirtifolium or Persian shallot also known as (moosir) is a native plant growing in some parts of Iran (Ashrafi et al., 2004; Fritsch et al., 2006), used as a dietary ingredient or medicinal remedy. It contains several alkannin derivatives, such as β-dimethylacrylalkannin, teracrylalkannin and isovalerylalkannin + α-methyl-n-butylalkannin (Ghahreman, 1984) and also have allicin, ajoene and other organosulfides (Mozaffarian, 1996; Rubatzky and Yamaguchi, 1997 and Jafarian et al., 2003). *A. hirtifolium* possess a variety of pharmacological properties. The aqueous extract has shown to have antibacterial activities (Ashrafi et al., 2004), as well as immunomodulatory effects (Kuttan, 2000), and has been used to suppress *Trichomonas vaginalis* growth (Taran et al., 2006). In addition, it has been used in treatment of rheumatic and inflammatory disorders (Jafarian et al., 2003). Recently, Barile and colleagues have shown that another plant of this family, *A. minutiflorum* has significant antimicrobial activity against a variety of fungals and bacterial strains (Barile et al., 2007).

Herein, we have explored the wound healing effects of topically applied *A. hirtifolium* extracts on full-thickness open wounds in rat.

Materials and methods

Herbal extract preparation

Persian shallot (*A. hirtifolium)* bulbs were collected from Khansar area in Isfahan province in early summer 2008. The specimen was identified and approved by Department of Biology, Tehran University, Iran. The bulbs were washed in tap water and, cut into small slices, air dried and ground into powder using a blender. The extract was made using 100 gr percolated powder in 250 ml distilled water mixed with 250 ml Alcohol 96% (1:1) and kept at 4° C for 24 hours. The solution filtered through cotton cloth for three times, left under the hood until dryness, powdered and stored at 4 ºC for later use (Polasek et al., 2007).

Preparation of treatment and control gels

For preparation of treatment gel (80mg/ml), 8gr of dry extract along with 3gr Methyl cellulose dissolved in 100 ml distilled water to form a gel-like mixture. The similar gel was made of Methyl cellulose alone for use as control agent.

Animals and experimental set-up:

Four adult albino rats weighing 165±35g were purchased from Razi Vaccine and Serum Research Institute, Mashhad, Iran. They were kept in standard conditions, in a temperature and light-controlled environment with free access to standard laboratory rodent food and fresh drinking water.

Anesthesia was induced by intramuscular (IM) injection of a combination of ketamine (40 mg/kg) and xylazin (10 mg/kg). Four full-thickness circular wounds (8.75 mm diameter) were made by biopsy punch on the dorsal area of each animal. The right side wounds were treated as experimental groups, while left side wounds were treated as control groups. One day after wounding, treatment gel was applied topically to the experimental wounds (100 mg/kg), while control groups were treated with the extract-free gel. The treatment was repeated once a day for 12 days. At days 1, 3, 6, 9 and 12, digital photos were taken from wounds. Scion image software was used to measure the percentage of wound contraction, epithelialisation and speed of wound healing. The following formulate were used:

Wound contraction:

1. Wound size at day (x) ^{mm2}/ wound size at day (0) ^{mm2} x 100= percentage wound size at day(x) compared to wound size at day (0)

2. Percentage wound size at day(x) compared to day (0) -100
= percentage wound contraction

Wound epithelialisation:

Size of epithelialisation area at day (x) ^{mm2}/Size of the wound at day (x) ^{mm2}/t x 100= percentage epithelialisation

Wound healing:

1. Granulation tissue at day (x) ^{mm2}/ wound size at day (0) ^{mm2} x 100= percentage non-healed area compared to the wound size at day (0)

2. Percentage non-healed area compared to the wound size at day (0) -100 = percentage healing

Statistical analysis

Effects of time on wound healing, epithelialization and contraction was examined using repeated measurement ANOVA, including time as fixed factor and rats as random factor. Statistical analysis was performed using the SPSS 9 program for Windows (SPSS Inc. Chicago IL, USA). A value of $p<0.05$ was considered significant.

Results

The wounds treated with *A. hirtifolium* extract exhibit noticeable healing improvement from day 6, healed faster and closed in shorter time than wounds in control group. All wounds in treatment group were completely healed within 11 to 12 days while it took about 13 to 14 days in control group (Figure 1).

A statistically significant difference was seen only in wound epitheliazation between treatment and control group during this study ($p<0.05$) (Figure 2), while there was no significant difference in wound contraction and wound healing between these two groups (Figures 3 and 4).

Discussion

The wound healing process is to reduce the pain and closing the wound as soon as possible to accelerate recovery of injured tissue with minimum cosmetic defects. In brief, it has three phases including inflammation, cellular proliferation and remodeling (Reddy et al., 2002).

The potential of traditional medicine to manage wound healing has been considered in some communities for thousands of years (Krishnan, 2006). *A. hirtifolium* is used as a herbal medicine in folklore. According to author's knowledge, no information is available about the wound healing activity of

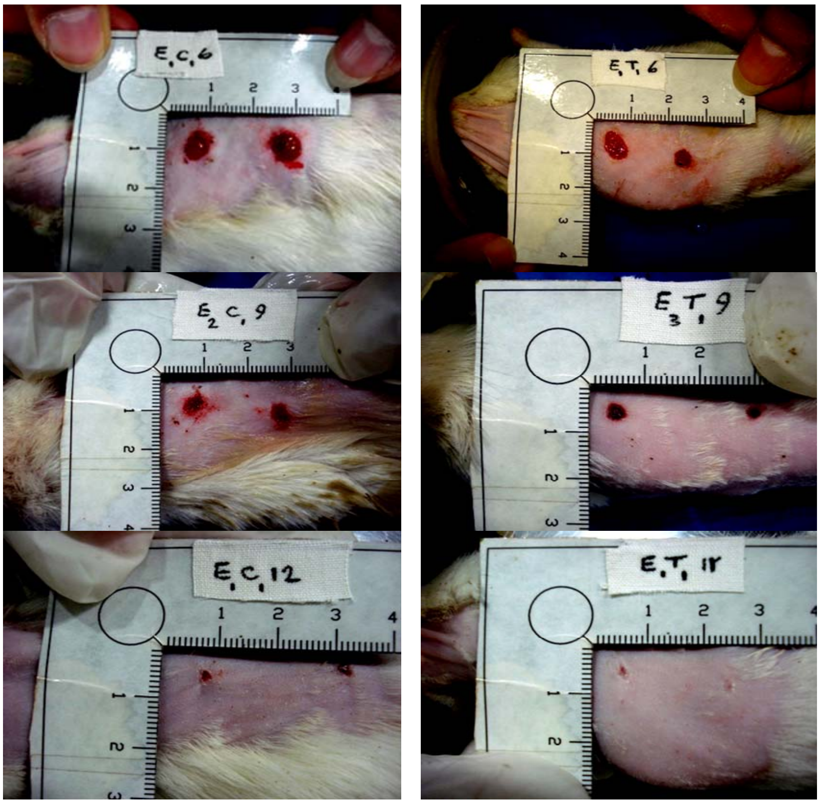


Figure1: Wound healing in animals treated with solution contain *A. hirtifolium* extract (right) and treated with Methyl cellulose alone (left), in day 6 (up), day 9 (middle) and day 12 (bottom).

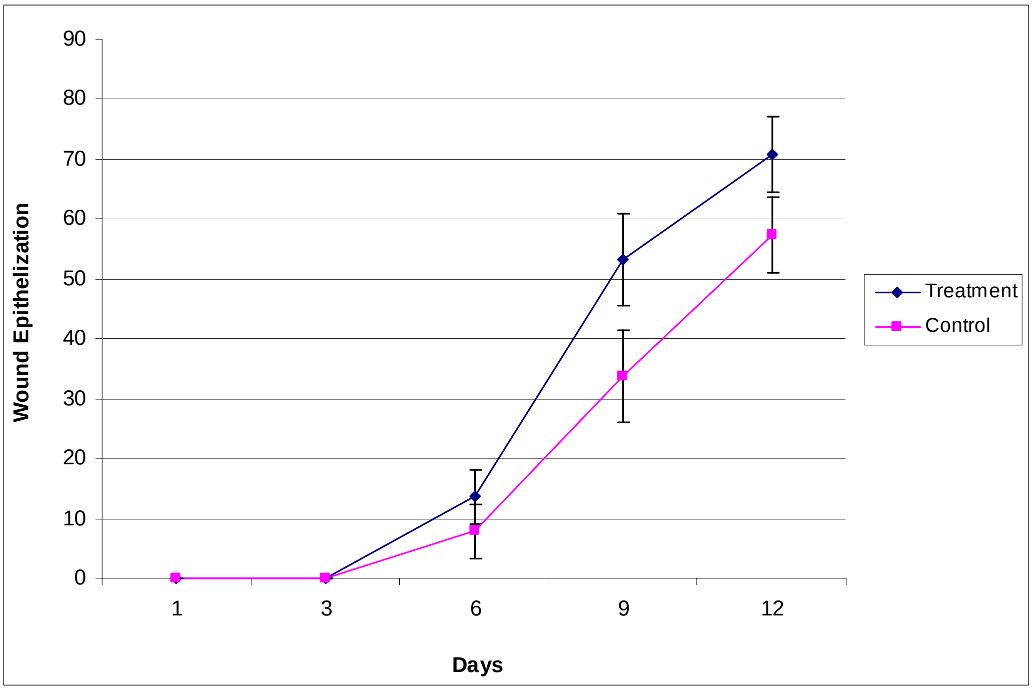


Figure 2: Percentage of wound epitheliazation in treatment and control wounds

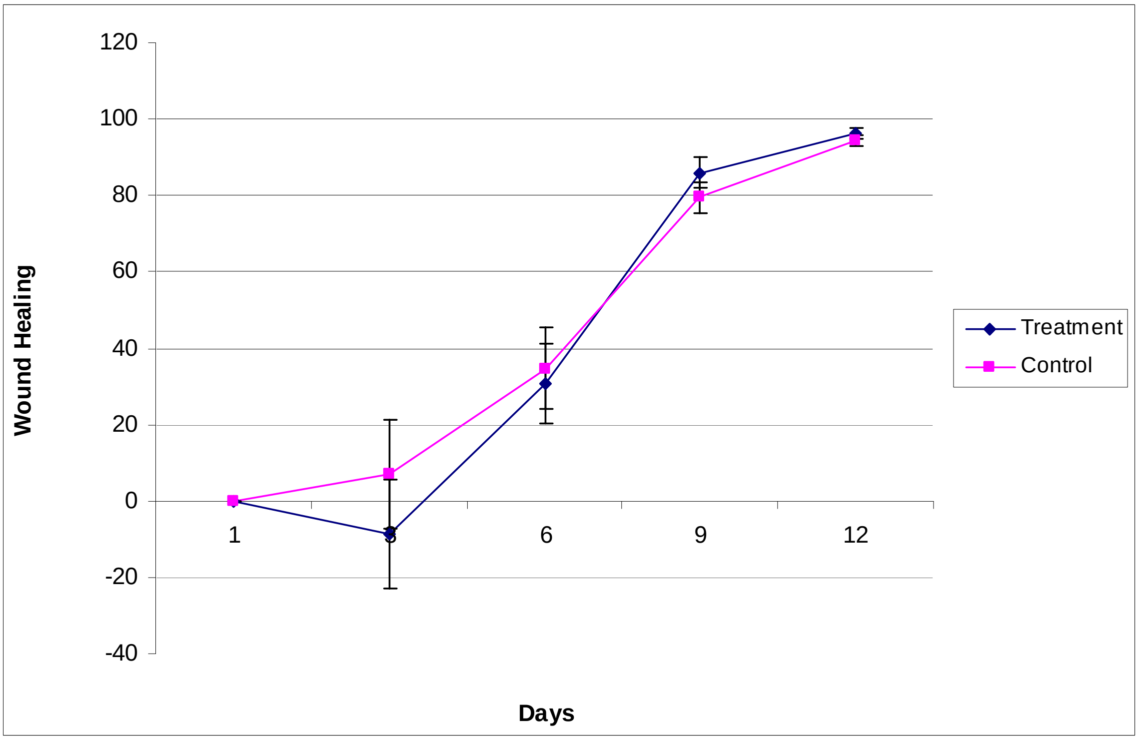


Figure 3: Percentage of wound healing in treatment and control wounds

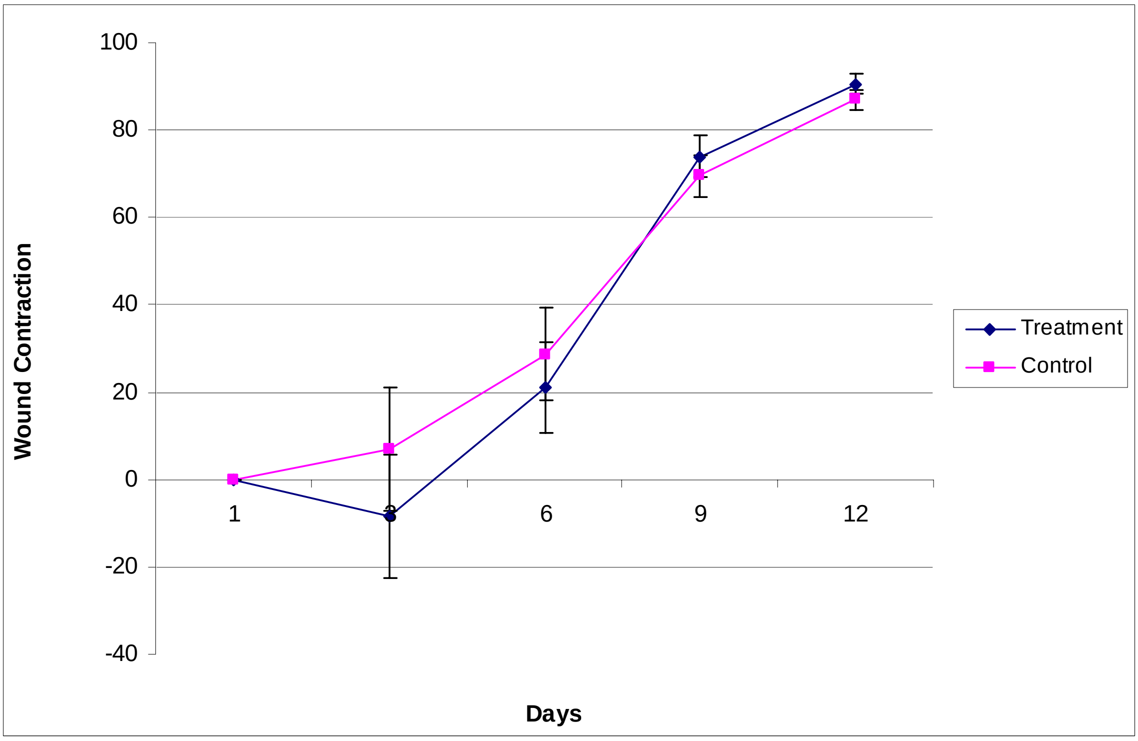


Figure 4: Percentage of wound contraction in treatment and control wounds

this plant. Therefore, the present study may be the first to evaluate the effects of *A. hirtifolium* on wound healing.

The results of this study revealed that *A. hirtifolium* significantly affects wound epithelialisation, as the first step of wound healing by which proliferation and migration of epidermal cells take place (Shukla and Mossman, 2008). The observed improvement in rate of wound closure, healing and reduction in healing time might be due to enhanced epithelialisation.

A. hirtifolium belongs to the same genus as *A. sativum* (garlic). Several reports have been shown that garlic is rich of allicin (diallyl thiosulfinate) (Panus, 2008), allicin has also been in *A.hirtifolium*, quantified in the amount of 3.4± 0.1 gr^{-1} (Ghodrati et al., 2008; Ghodrati et al., 2009). Sardari and colleagues have reported that topical application of allicin has no significant effect on wound contraction, epithelialization and healing in full-thickness wound in dogs (Sardari et al., 2006). Therefore, it seems that allicin is not responsible for wound epthelialization. However, non-significant effect of *A. hirtifolium* on wound contraction and healing in the present study is similar to what sardari and colleagues have shown about allicin. In addition to allicin, *A. hirtifolium* contains several secondary metabolites such as alliin, alliinase, S-allyl-cysteine (SAC), diallyldisulphide (DADS), diallyltrisulphide (DATS), and methylallyltrisuphide (Block et al., 1992), which may be responsible for its effect on epithelialisation lonely or in combination. Although it is unlikely that experimental animals (rats and dogs) make differences in results but we should consider that, different animals may respond to plant extract in a different way.

The results of this study raise the possibility that topical application of *A. hirtifolium* can accelerates or improves the healing of small granulating wounds by encourage the epitheliazation and may be clinically useful in management of open wounds.

Further studies should perform with a variety of doses of *A. hirtifolium* extract to establish the appropriate dosage. Also use of the fractionation of active compounds may lead to better result in the process of wound healing activity of this herbal medicine.

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ارزیابی ماکروسکوپی تاثیر عصاره موسیر (*Allium hertifolium*) بر ترمیم زخم در رت

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چکیده

زخم نوعی تخریب لایه بیرونی پوست می باشد. تا کنون روشهای مختلفی از جمله تجویز داروهای موضعی و خوراکی و یا حتی داروهای گیاهی به منظور تسریع در روند التیام زخم استفاده گردیده اند. گیاه موسیر فارسی با نام علمی *Allium hirtifolium* که متعلق به همان خانواده ای است که جنس سیر در آن قرار دارد، به طور طبیعی در بخش های مختلفی از ایران رشد می کند. در این مطالعه، روند التیام زخم ایجاد شده و تاثری عصاره آبی الکلی گیاه موسیر مورد بررسی قرار گرفته است. ابتدا چهار زخم تمام ضخامت دو طرفه (۲ زخم در هر سمت) در منطقه میانی چهار رت بالغ از نژاد آلبینو با وزن ۳۵ ± ۱۶۵ گرم تحت بیهوشی عمومی ایجاد شد. زخم های سمت راست به عنوان گروه تحت درمان و زخم های سمت چپ به عنوان کنترل در نظر گرفته شدند. یک روز پس از جراحی، زخم های گروه درمانی به مدت ۱۲ روز مورد تجویز مخلوطی ژل مانند به نسبت ۱:۱ از عصاره موسیر و متیل سلولز به طور موضعی (۱۰۰ میلی گرم / کیلوگرم / روز) قرار گرفتند، در حالیکه زخم های گروه کنترل با ژل فاقد عصاره پوشانده شدند. در روزهای ۱، ۳، ۶، ۹ و ۱۲، عکس های دیجیتال زخمها با استفاده از نرم افزار Scion image مورد بررسی درصد انقباض زخم، اپیتلیزاسیون و سرعت بهبود قرا گرفت. نتایج حاکی از آن بود که موسیر از طریق افزایش میزان اپیتلیزاسیون روند التیام زخم را سرعت می بخشد. نتیجه مطالعه حاضر می تواند راه را برای استفاده از اثرات مفید عصاره موسیر در درمانهای بالینی هموار سازد.

واژگان کلیدی : موسیر ، اپیتلیزاسیون ، عصاره، بهبود زخم